

EPIDEMIOLOGICAL ASPECTS OF LYMPHOGRANULOMA VENEREUM*

A STUDY OF A GROUP OF PROSTITUTES

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The incidence of lymphogranuloma venereum (L.G.V.) or Nicolas Favre disease has decreased in recent years unlike syphilis and gonorrhoea, which have tended to increase. An analysis of the graphs given by Haustein (1927) shows that the incidence of these diseases has important variations and, even before the appearance of arsenic, bismuth, and antibiotics, syphilis had periods of lowered incidence, like other endo-epidemic diseases.

It seems important to inquire into the causes of the decreased incidence of L.G.V. and to ascertain if new epidemic cycles of the disease are probable. We thought that the study of prostitutes might tell us something about the present epidemiological features of L.G.V., including the possible reservoirs from which the infection might spread.

The epidemiological study of L.G.V. depends on two tests using specific antigens: Frei's intradermal reaction and the complement-fixation test.

Previous studies, using the serological method alone or in association with Frei's test, have been carried out on normal individuals as well as those suffering from L.G.V. The results of these studies show that the serological test is more sensitive, becomes positive earlier, and that, according to the antibody titre, it can give some indication of the stage of the disease (Grace and Rake, 1943; Packer and Dulaney, 1947; Greaves and Taggart, 1953; King, Barwell, and Catterall, 1956; and Alergant, 1957).

Beeson and Miller (1944) in the U.S.A., who made a serological study of 879 individuals not suspected of suffering from L.G.V., found a positive result in an appreciable number: 11·6 per cent. in white men,

12·1 per cent. in white women, 44·8 per cent. in coloured men, and 39·7 per cent. in coloured women. These workers suggest that the positive results probably indicate that the virus persists in the body and so continues to stimulate the production of antibody.

The complement-fixation test has also been used to assess the therapeutic effect of certain antibiotics in the late stages of the disease (Goldberg and Banov, 1956).

We also wished to ascertain whether the relationships between the antigens of *B. anitratum* and the antibodies present in cases of ornithosis (Volkert and Mathiesen, 1956, 1958; Mathiesen and Volkert, 1957, 1958; Mathiesen, 1957), would still hold good in the presence of antibodies corresponding with more probability to L.G.V. virus.

Material and Methods

Sera.—The sera were obtained from fifty prostitutes, registered and unregistered, in the city of Lisbon. Their ages ranged from 22 to 61 years, and the stated period of prostitution from one week to 35 years.

Once the serum was separated from the coagulum, it was kept in the deep-freezer at -25° C. Before the sera were tested they were inactivated at 60° C. for 20 minutes. In all the women studied, quantitative serological examinations for syphilis were also made; in none did the titre exceed the dilution of 1 : 8.

Complement-fixation Test with Antigen from the Virus of Lymphogranuloma Venereum.—The reaction was carried out in tubes, using 0·2 ml. of each of the reagents. The diluting fluid was veronal buffer (Mayer, Osler, Bier, and Heidelberger, 1946), and sheep red cells were employed at a concentration of 2 per cent. Two units of haemolytic serum, titrated for each series of tests, and two units of

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commercial antigen (Lygranum*) prepared from virus cultivated in egg-yolk sac were used. The complement was titrated in the presence of the antigen, with a period of primary incubation identical to that used in the test. We used 2 exact units of complement. Sera were first tested at a dilution of 1 : 5. Sera positive at this dilution were later tested with 2-fold serial dilutions, starting at 1 : 8. Pipettes were changed for each dilution.

For each dilution of serum, a control antigen prepared from non-infected egg-yolk sac was used in addition to the specific antigen. Controls of the anti-complementary activity of the serum, in the absence of the antigen, were routinely used. Fixation was effected in a water-bath at 37° C. for 75 minutes. Controls of the complement were also made at 2, 1, and 0.5 units in the presence of the antigen, thus constituting simultaneous controls of the anti-complementary strength of the latter.

After adding the components of the haemolytic system and effecting the secondary incubation at 37° C. for a maximum of 30 minutes, readings of the reaction were made. A reaction was considered to be positive when there was more than 50 per cent. inhibition of haemolysis.

Complement-Fixation Reaction for *B. anitratum*.—The technique followed was, in general, similar to the previous one. The soluble antigen (kindly provided by Dr. M. Volkert of the Statens Serum-Institut of Copenhagen) was prepared from agar cultures of *B. anitratum*, using double centrifugation and boiling for 30 minutes. Primary incubation was effected during the night in the icebox at -4° C. The processes of titration and the controls employed were identical with those previously described.

Frei's Intradermal Reaction.—This was carried out in 32 women only, using either "Lygranum" or an antigen prepared by us from the pus of a patient suffering from L.G.V. The latter antigen was prepared by Frei's original technique, diluting the pus with five times its volume of isotonic chloride solution and subsequently heating it to 60° C. for 2 hours and, on the next day, for another hour at the same temperature. Thereafter tests of its bacterial sterility and of the inactivation of the virus were made, using for the latter mouse inoculation by the intracerebral route, and of the yolk-sac of chick embryo. The activity of the antigen obtained by us was verified in patients on whom the "Lygranum" test was used simultaneously. Identical responses to the two antigens were obtained in all cases.

Frei's reaction was performed by intradermal injection on the anterior surface of the forearm of 0.1 ml. of the specific antigens and of 0.1 ml. of a control antigen prepared from normal yolk-sac. The reading was made after 48 hours, the reaction being considered positive when an infiltrated papule of at least 10 mm. was observed at the site of the specific antigen, while at the same time there was no response to the control.

* Supplied by Squibb Ltd.

Results

Complement-Fixation Reaction with Antigen from the Virus of L.G.V.—The results obtained are shown in Table I.

TABLE I
RESULTS WITH L.G.V. ANTIGEN

Number of Sera from Prostitutes Examined		No. of Positive Results in Various Dilutions of Sera		Percentage of Positive Results
Registered	27	$\left. \begin{array}{l} 1/5 \\ 1/8 \\ 1/16 \\ 1/32 \end{array} \right\}$	$\left. \begin{array}{l} 7 \\ 7 \\ 6 \\ 2 \end{array} \right\} 22$	81
Unregistered	23	$\left. \begin{array}{l} 1/5 \\ 1/8 \\ 1/32 \\ 1/64 \end{array} \right\}$	$\left. \begin{array}{l} 4 \\ 2 \\ 1 \\ 1 \end{array} \right\} 8$	35
Total	50	30		60

The average age of the registered prostitutes was 42 years, and that of the unregistered 28 years. The average period of prostitution admitted by the registered was 17 years and by the unregistered 6 years.

Complement-Fixation Reaction with *B. anitratum* Antigen.—The results obtained are shown in Table II.

TABLE II
RESULTS WITH *B. ANITRATUM* ANTIGEN

Number of Sera from Prostitutes Examined		No. of Positive Results in Various Dilutions of Sera	Percentage of Positive Results
Registered	27	$\left. \begin{array}{l} 1/5 \quad 6 \\ 1/8 \quad 8 \\ 1/16 \quad 2 \end{array} \right\} 16$	59
Unregistered	23	$\left. \begin{array}{l} 1/5 \quad 2 \\ 1/8 \quad 4 \\ 1/16 \quad 1 \\ 1/32 \quad 1 \end{array} \right\} 8$	35
Total	50	24	48

Comparison between the Complement-Fixation Test with the Antigen of L.G.V. and with that of *B. anitratum*.—This is demonstrated in Table III.

TABLE III
RESULTS WITH L.G.V. AND *B. ANITRATUM* ANTIGEN COMPARED

Number of Sera	Positive Results			Positive Results with <i>B. anit.</i> as Percentage of Those with L.G.V.
	With L.G.V. Antigen	With <i>B. anit.</i> Antigen	With L.G.V. and <i>B. anit.</i> Antigen	
50	30	24	20	67

The titres of the sera which were positive with the two antigens were similar, differing at most by two dilutions in favour of those belonging to the antigens

of L.G.V. Of the ten sera which were positive with the "Lygranum" antigen at a dilution of 1/16 or more, none was negative with the *B. anitratum* antigen.

Four sera were positive with *B. anitratum* antigen but not with the L.G.V. antigen. None had a titre over 1/8.

Partial Study of Frei's Reaction.—Of 32 prostitutes, ten (31 per cent.) gave a positive Frei reaction (Table IV). The L.G.V. antibody titre in women who had a positive Frei's reaction varied from 1/5 to 1/64.

TABLE IV
FREI'S REACTION

Number of Prostitutes Examined	Frei Test Positive	Frei Test Positive Complement-Fixation Test for L.G.V. Positive	Frei Test Negative: Complement-Fixation Test for L.G.V. Positive
32	10 (Percentage of Total 31)	10 (Relative Percentage 100)	11 (Relative Percentage 52)

Comment

Although our study has been restricted to only fifty individuals, its usefulness may be enhanced by the fact that all were prostitutes and thus specially liable to venereal infection. No similar study of L.G.V. has been found in the literature.

The first important point is the cross-reaction existing between the virus causing L.G.V. and the other components of the psittacosis-lymphogranuloma venereum group. However, because the data for our study were obtained from prostitutes, the group antibodies which they presented were more likely to correspond to past infection with the virus of lymphogranuloma venereum, a venereal disease. In order to clear up this problem it would be interesting to compare the existence of antibodies in other population groups. In Portugal, of 147 patients suspected of having atypical pneumonia and therefore likely to include cases of ornithosis (Pinto and de Sousa, 1954), only ten (8 per cent.) were found to be positive. In New Zealand, a study carried out on 204 normal people showed four positive reactions for L.G.V., with minimal titres of 1 : 5, which gives the very low percentage of 1.9 per cent. (Manning and Reid, 1958).

It may be asked whether positive reactions represent only a serological residuum of past infection or

whether they indicate the existence of the virus in the body? Beeson and Miller (1954) consider that dilutions of 1/5 or more indicates a persistence of the virus in the patient.

In our study, the percentage (60 per cent.) of the titre of 1 : 5 or more seems to be very significant. Even if we consider only the titres of 1:16 or more as an expression of active, unnoticed disease, the results obtained are still important (20 per cent.). These data agree with those observed in the clinics where patients in the late stages of the disease appear some years after the resolution of the primary infection.

From the epidemiological point of view, our results could suggest that these women with high titres may be reservoirs of virus and able to disseminate the infection. It seems possible that it is from these cases, too, that the anorectal complication of the disease more frequently arises. It is interesting to note the larger percentage (81 per cent.) of positive results in the registered prostitutes—older in age as well as in the profession—than in those unregistered (35 per cent.), though amongst them, too, the virus is already disseminated.

The results of Frei's reaction—ten out of 32 were found to be positive—confirms the greater sensitivity of the serological test over the intradermal reaction, which has already been pointed out by other workers.

Respecting the cross-reactions with *B. anitratum*, although the percentage obtained by us (67 per cent.) is slightly less than that reported by Danish workers in relation to cases of ornithosis, our results seem strongly to favour the existence of an antigenic relationship between the virus of L.G.V. and *B. anitratum*.

Summary

In a serological study, complement-fixing antibodies against L.G.V. antigen were found in 60 per cent. of sera from fifty prostitutes.

The number of positive results was higher among the registered and older individuals (81 per cent.) than among the unregistered and younger subjects (35 per cent.).

In 24 sera, complement-fixing antibodies against *B. anitratum* antigen were also found; twenty of these were positive for both antigens.

The Frei test was positive in ten out of 32 individuals; all ten had complement-fixing antibodies in their sera in titres from 1 : 5 to 1 : 64. The Frei positives amounted to only 52 per cent. of the complement-fixing antibody positives.

REFERENCES

- Alergant, C. D. (1957). *Brit. J. Vener. Dis.*, **33**, 47.
- Beeson, P. B., and Miller, E. S. (1944). *Amer. J. publ. Hlth*, **34**, 1076.
- Grace, A. W., and Rake, G. (1943). *Arch. Derm. Syph. (Chicago)*, **48**, 619.
- Greaves, A. B., and Taggart, S. R. (1953). *Amer. J. Syph.*, **37**, 273.
- Goldberg, J., and Banov, L. (1956). *Brit. J. vener. Dis.*, **32**, 37.
- Haustein, H. (1927). In "Handbuch der Haut-und Geschlechtskrankheiten", ed. J. Jadassohn, vol. 22, Springer, Berlin.
- King, A. J., Barwell, C. G., and Catterall, R. D. (1956). *Brit. J. vener. Dis.*, **32**, 209.
- Manning, J. D., and Reid, J. D. (1958). *Amer. J. clin. Path.*, **29**, 430.
- Matthiesen, M. (1957). *Acta path. microbiol. scand.*, **41**, 247.
- and Volkert, M. (1957). *Ibid.*, **41**, 135.
- — (1958). *Ibid.*, **44**, 440.
- Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberg, M. (1946). *J. exp. Med.*, **84**, 535.
- Packer, H., and Dulaney, A. D. (1947). *Amer. J. Syph.*, **31**, 69.
- Pinto, M. R., and Plácido de Sousa, C. (1954). *J. Soc. Cienc. med. Lisboa*, **118**, 8.
- Volkert, M., and Matthiesen, M. (1956). *Acta path. microbiol. scand.*, **39**, 117.
- — (1958). *Ibid.*, **44**, 278.

Aspects épidémiologiques de la lymphogranulomatose vénérienne

Résumé

Dans une étude sérologique, les anticorps fixant l'alexine contre l'antigène de la L.G.V. étaient présents dans 60 % des sérums de 50 prostituées.

Le nombre de résultats positifs était plus élevé chez les vieilles prostituées enregistrées (81 %) que chez les jeunes non enregistrées (35 %).

Dans 24 sérums, il y avait aussi des anticorps fixant l'alexine contre l'antigène *B₁ anitratum*. 20 de ces sérums étaient positifs pour les deux antigènes.

Le test de Frei était positif chez 10 femmes sur 32; le titre des anticorps fixant l'alexine du sérum de ces 10 prostituées variait de 1:5 à 1:64. Le test de Frei n'était positif que dans 52 % des cas où les anticorps fixant l'alexine étaient positifs.